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(54) Title: NOVEL CYTOKINE RECEPTORS			
(57) Abstract A novel polypeptide that is believed to be a novel type 1 cytokine receptor has been identified in both mice and in humans and the corresponding cDNA sequences have been obtained. There is a high degree of conservation of amino acid between the human and murine polypeptides, indicating that this receptor is functionally important. Polypeptides within the scope of the present invention may be useful in treating cancer, obesity and immune or developmental disorders. They may also be useful in screening.			

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Novel Cytokine Receptors

The present invention relates *inter alia* to novel molecules believed to be cytokine receptors and to uses thereof.

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Cytokines and growth factors are secreted molecules controlling important cell functions such as proliferation, differentiation and survival as well as tissue development. These signalling molecules exert their effects via specific receptors located on the target cell surface. These receptors are grouped into families according to both structural and amino acid sequence similarities. The cytokine receptor superfamily is composed of the receptors for many growth factor families including interferon, TNF and haematopoietic growth factors. The largest subclass in this family is that of the type I cytokine receptors, a group characterized by the presence of a conserved extracellular region of approximately 200 amino acids containing two fibronectin type III folds. This region, known as the haematopoietin receptor module, has been shown to play an essential role in receptor/ligand binding and receptor/receptor dimerization. It is characterized by four conserved cysteine residues in the first domain and a W-S-x-W-S motif in the second domain.

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Receptor chains in this subclass typically form part of a multicomponent complex which includes both ligand binding and signalling subunits, of which the latter is typically a member of several receptor complexes. These characteristics account for much of the pleiotropy and redundancy amongst cytokines. One such example is amongst certain members of the interleukin-6 (IL-6) related cytokines (IL-6, ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), oncostatin M (OSM) and cardiotrophin-1 (CT-1)), where overlapping function between these cytokines can be accounted for by the presence of gp130, a signal transducing molecule present in the respective receptor complexes. In addition, ligand binding chains in the receptor complexes (such as IL-6R α , CNTF-R and IL-11R α) generally have short or even absent intracytoplasmic

regions, whereas gp130 has a longer intracytoplasmic tail which is involved in the activation of the JAK-STAT pathway. In fact, soluble forms of these receptor subunits can render cells sensitive to the appropriate cytokine provided the necessary signal transducing chain is expressed on the cell surface. Thus, the specific cytokine binding chains of these cytokine receptors can function either as membrane anchored or soluble proteins.

The present invention is based upon the identification and characterisation of a previously unknown mouse molecule and of a previously unknown human molecule, both of which are believed to be new type 1 cytokine receptors.

According to the present invention there is provided a polypeptide, which:

- a) has the amino acid sequence of amino acids 38 to 422 shown in Figure 1 for hGBRI-ILR or which has the amino acid sequence shown in Figure 1 for mGBRI-ILR;
- b) has one or more amino acid deletions, insertions or substitutions relative to a polypeptide as defined in a) above, but has at least 40% amino acid sequence identity therewith; or
- c) is a fragment of a polypeptide as defined in a) or b) above, which is at least 10 amino acids long.

The term "polypeptide" is used herein in a broad sense to indicate that a particular molecule comprises a plurality of amino acids joined together by peptide bonds. It therefore includes within its scope substances which may sometimes be referred to in the literature as peptides, polypeptides or proteins.

A polypeptide of the present invention preferably incorporates a haematopoietin domain since this is believed to be important in ligand / receptor and receptor / receptor complex formation.

Various aspects of the present invention will now be considered in order that its full scope can be appreciated.

5 Polypeptides of the present invention may be produced by techniques known to those skilled in the art. For example, gene-cloning techniques may be used to provide a nucleic acid sequence encoding such a polypeptide. (Gene-cloning techniques are discussed in greater detail later on in relation to nucleic acid molecules of the present invention.) Alternatively, chemical synthesis techniques may be used to produce polypeptides of the
10 present invention. Such techniques generally utilise solid-phase synthesis. Chemical synthesis techniques that allow polypeptides having particular sequences to be produced have now been automated. Apparatuses capable of chemically synthesising polypeptides are available, for example, from Applied Biosystems. If desired, short polypeptides can be synthesised initially and can then be ligated to produce longer polypeptides.

15 A polypeptide of the present invention may be provided in substantially pure form. Thus it may be provided in a composition in which it is the predominant polypeptide component present. (It may be present e.g. at a level of more than 50%, of more than 75%, of more than 90%, or even of more than 95%; said levels being determined on a
20 weight/weight basis with respect to the total polypeptide content of the composition.)

As explained previously, a polypeptide of the present invention either:

- 25 a) has the amino acid sequence of amino acids 38 to 422 shown in Figure 1 for hGBRI-ILR or has the amino acid sequence shown in Figure 1 for mGBRI-ILR;
- b) has one or more amino acid deletions, insertions or substitutions relative to a polypeptide as defined in a) above, but has at least 40% amino acid sequence identity therewith; or

- c) is a fragment of a polypeptide as defined in a) or b) above, which is at least 10 amino acids long.

In order to appreciate the present invention more fully, polypeptides within the scope of each of a), b) and c) above will now each be discussed in greater detail.

Polypeptides within the scope of a)

A polypeptide within the scope of a) may consist of the amino acid sequence of amino acids 38 to 422 shown in Figure 1 for hGBRI-ILR or of the amino acid sequence shown in Figure 1 for mGBRI-ILR. Alternatively it may have an additional N-terminal and/or an additional C-terminal amino acid sequence.

Additional N-terminal or C-terminal sequences may be provided for various reasons. Techniques for providing such additional sequences are well known in the art. These include using gene-cloning techniques to ligate together nucleic acid molecules encoding polypeptides or parts thereof, followed by expressing a polypeptide encoded by the nucleic acid molecule produced by ligation.

Additional sequences may be provided in order to alter the characteristics of a particular polypeptide. This can be useful in improving expression or regulation of expression in particular expression systems. For example, an additional sequence may provide some protection against proteolytic cleavage. This has been done for the hormone somatostatin by fusing it at its N-terminus to part of the β galactosidase enzyme (Itakwa *et al.*, *Science* 198: 105-63 (1977)).

Additional sequences can also be useful in altering the properties of a polypeptide to aid in directing the polypeptide to a particular location. For example, a signal sequence may be present to direct the transport of the polypeptide to a particular location within a cell or to export the polypeptide from the cell (e.g. the amino acids 1 to 37 shown in Figure

1 for hGBRI-ILR may be used to provide a signal sequence, or another signal sequence may be present). Different signal sequences can be used for different expression systems.

Hydrophobic sequences may be provided to anchor a polypeptide in a membrane. Thus
5 the present invention includes within its scope both soluble and membrane-bound polypeptides. (Naturally occurring membrane-bound forms of the polypeptides identified in Figure 1 are believed to exist since large mRNA transcripts likely to encode such forms have been identified by the present inventors, as will be discussed later.) Membrane-bound polypeptides may be in hybrid form if desired. They may therefore
10 have a heterologous transmembrane and/or cytoplasmic domain. For example such domains may be derived from a human IL-13 receptor α chain. Transfected mammalian cells expressing membrane-bound polypeptides can be used for ligand screening and binding assays (e.g. for antibodies or other molecules binding to the receptor).

15 Another example of the provision of an additional sequence is where a polypeptide is linked to a moiety aiding in purification / identification, e.g. a moiety capable of being isolated by affinity chromatography. The moiety may be an epitope and the affinity column may comprise immobilised antibodies or immobilised antibody fragments that bind to said epitope (desirably with a high degree of specificity). The polypeptide can
20 then be eluted from the column by addition of an appropriate buffer and may be cleaved from the epitope. His₆, Glu₂ or 179 tags are preferred for use in purification / identification. Polypeptides comprising one or more such tags are therefore within the scope of the present invention.

25 A polypeptide may be linked to an antibody or to a part thereof. For example it may be linked to an F_c portion. This results in a molecule with good stability that can be used both *in vitro* and *in vivo*. It may be linked to a part of an antibody that binds to a particular epitope of it is desired to target that epitope.

In the case of the of the amino acid sequence shown in Figure 1 for mGBRI-ILR, additional amino acids may be provided to result in an amino acid sequence closer in length to the length of the 422 amino acid polypeptide shown in Figure 1 for hGBRI-ILR. For example, an additional two amino acids immediately N-terminal to the amino acid sequence shown in Figure 1 for mGBRI-ILR polypeptide may be provided. (These may be A and H, as is the case for hGBRI-ILR). A signal sequence may also/alternatively be provided.

It should be noted that additional N-terminal or C-terminal sequences may be present simply as a result of a particular technique used to obtain a polypeptide of the present invention and need not provide any particular advantageous characteristic.

Polypeptides within the scope of b)

Turning now to the polypeptides defined in b) above, it will be appreciated by a person skilled in the art that these are variants of the polypeptides given in a) above.

The skilled person will appreciate that various changes can sometimes be made to the amino acid sequence of a polypeptide which has a desired property to produce variants (often known as "muteins") that still have said property. Such variants of the polypeptides described in a) above are within the scope of the present invention and are discussed in greater detail below in sections (i) to (iii). They include allelic and non-allelic variants.

(i) Substitutions

An example of a variant of the present invention is a polypeptide as defined in a) above, apart from the substitution of one or more amino acids with one or more other amino acids.

The skilled person is aware that various amino acids have similar characteristics. One or more such amino acids of a polypeptide can often be substituted by one or more other such amino acids without eliminating a desired property of that polypeptide.

5 For example, the amino acids glycine, alanine, valine, leucine and isoleucine can often be substituted for one another (amino acids having aliphatic side chains). Of these possible substitutions it is preferred that glycine and alanine are used to substitute for one another (since they have relatively short side chains) and that valine, leucine and isoleucine are used to substitute for one another (since they have larger aliphatic side chains which are hydrophobic).

Other amino acids that can often be substituted for one another include:

phenylalanine, tyrosine and tryptophan (amino acids having aromatic side chains);

lysine, arginine and histidine (amino acids having basic side chains);

15 aspartate and glutamate (amino acids having acidic side chains);

asparagine and glutamine (amino acids having amide side chains);

and cysteine and methionine (amino acids having sulphur containing side chains).

20 Substitutions of this nature are often referred to as "conservative" or "semi-conservative" amino acid substitutions.

(ii) Deletions

Amino acid deletions can be advantageous since the overall length and the molecular weight of a polypeptide can be reduced whilst still retaining a desired property. This can enable the amount of polypeptide required for a particular purpose to be reduced. For example if the polypeptide is to be used in medicine, dosage levels can be reduced.

(iii) Insertions

Amino acid insertions relative to a polypeptide as defined in a) above can also be made. This may be done to alter the nature of the polypeptide (e.g. to assist in identification, purification or expression, as explained above in relation to fusion proteins).

5 Polypeptides incorporating amino acid changes (whether substitutions, deletions or insertions) relative to the sequence of a polypeptide as defined in a) above can be provided using any suitable techniques. For example, a nucleic acid sequence incorporating a desired sequence change can be provided by site-directed mutagenesis. This can then be used to allow the expression of a polypeptide having a corresponding
10 change in its amino acid sequence.

Whatever amino acid changes may be made, preferred polypeptides of the present invention have at least 40% amino acid sequence identity with the amino acid sequence of amino acids 38 to 422 shown in Figure 1 for hGBRI-ILR or with the amino acid
15 sequence shown in Figure 1 for mGBRI-ILR. More preferably the degree of sequence identity is at least 50% or at least 75%. Sequence identities of at least 90% or of at least 95% are most preferred.

For the purposes of the present invention sequence identity (whether amino acid or
20 nucleic acid) can be determined by using the "BESTFIT" program of the Wisconsin Sequence Analysis Package Genetics Computer Group version 8.0.

Where high degrees of sequence identity are present there may be relatively few differences in amino acid sequence. Thus for example there may be less than 20, less
25 than 10, or even less than 5 differences.

Polypeptides within the scope of c)

As discussed *supra*, it is often advantageous to reduce the length of a polypeptide. Feature c) of the present invention therefore covers fragments of the polypeptides a) or

b) above which are at least 10 amino acids long. Desirably these fragments are at least 20, at least 50 or at least 100 amino acids long. Fragments may be useful, for example, in raising antibodies against particular antigens. They may also be useful in studying functionally important domains of a full length polypeptide. Thus, for example, a fragment comprising all or part of a haematopoietin receptor module may be provided.

Uses of Polypeptides

A) Medical Uses

Polypeptides of the present invention may be used in medicine. Preferred treatments are human treatments, although veterinary treatments are not excluded. The treatment may be prophylactic or may be in respect of an existing condition.

Various polypeptides of the present invention may be useful either as agonists or as antagonists. Agonists will up-regulate a biological function of a naturally occurring receptor, whereas antagonists will down-regulate such a function. Whether or not a given polypeptide acts as an agonist or an antagonist of a particular biological function can be determined by a skilled person using an appropriate assay procedure.

Antagonists may be useful in treating disorders associated with an overexpression of a cytokine or with the expression of a moiety having a level of cytokine activity higher than normal. In view of the homology with IL6-R, antagonists of one or more of the functions of receptors of the present invention may be useful in treating disorders associated with high levels of cell proliferation (e.g. in treating cancer). Antagonists may also be useful in treating immune disorders, weight disorders and / or developmental disorders. In particular they may be useful in treating obesity (in view of homology of the polypeptides shown in Figure 1 with the leptin receptor), inflammation, septic shock, AIDS and disorders of embryonic development.

Agonists may be useful in treating disorders associated with an underexpression of a cytokine or with the expression of a moiety having a level of cytokine activity lower than normal. They may be useful in treating disorders associated with low levels of cell proliferation. Agonists may also be useful in treating immune disorders, weight disorders and / or developmental disorders. In particular they may be useful in treating obesity (in view of homology of the polypeptides shown in Figure 1 with the leptin receptor), inflammation, septic shock, AIDS and disorders of embryonic development.

Antagonists or agonists may therefore be used in the manufacture of a medicament for the treatments mentioned above.

The medicament will usually be supplied as part of a pharmaceutical composition, which may include a pharmaceutically acceptable carrier. This pharmaceutical composition will generally be provided in a sterile form in a sealed container. It may be provided in unit dosage form, will generally be provided in a sealed container, and can be provided as part of a kit. Such a kit is within the scope of the present invention. It would normally (although not necessarily) include instructions for use. A plurality of unit dosage forms may be provided.

Pharmaceutical compositions within the scope of the present invention may include one or more of the following: preserving agents, solubilising agents, stabilising agents, wetting agents, emulsifiers, sweeteners, colorants, odourants, salts, buffers, coating agents or antioxidants. They may also contain therapeutically active agents in addition to polypeptides of the present invention. They may be provided in controlled release form, e.g. so as to be effective over a period of at least a week or, more preferably, of at least a month.

A pharmaceutical composition within the scope of the present invention may be adapted for administration by any appropriate route, for example by the oral (including buccal or

sublingual), rectal, nasal, topical (including buccal, sublingual or transdermal), vaginal or parenteral (including subcutaneous, intramuscular, intravenous or intradermal) routes. Such a composition may be prepared by any method known in the art of pharmacy, for example by admixing one or more active ingredients with a suitable carrier under sterile conditions.

Dosages

Dosages of an active agent can vary between wide limits, depending upon the nature of the treatment, the age and condition of the individual to be treated, etc. and a physician will ultimately determine appropriate dosages to be used. A dosage may be repeated as often as appropriate. If side effects develop, the amount and/or frequency of the dosage can be reduced, in accordance with good clinical practice.

B) Diagnostics Uses

In addition to the medical uses discussed above, polypeptides of the present invention can be used in diagnosis. For example they can be used in binding studies to diagnose the presence or absence of a type 1 cytokine or to diagnose abnormalities in the level of such a cytokine.

C) Screening Uses

Polypeptides of the present invention can also be used in screening. For example soluble or membrane bound receptors / variants thereof may be used to screen for agents capable of binding thereto. Such agents may be the cytokines which normally bind to the receptors *in vivo*. Alternatively they may be agonists or antagonists of such cytokines and may be useful in treating one or more of the disorders discussed in A) above.

D) Uses in Raising or Selecting Antibodies

One further use of the polypeptides of the present invention is in raising or selecting antibodies.

The present invention therefore includes antibodies that bind to a polypeptide of the present invention. Preferred antibodies bind specifically to polypeptides of the present invention and can therefore be used to purify such polypeptides (e.g. they may be immobilised and used to bind to polypeptides of the present invention. The polypeptides may then be eluted by washing with a suitable eluent under appropriate conditions.)

Antibodies within the scope of the present invention may be monoclonal or polyclonal.

Polyclonal antibodies can be raised by stimulating their production in a suitable animal host (e.g. a mouse, rat, guinea pig, rabbit, sheep, goat or monkey) when a polypeptide of the present invention or a nucleic acid molecule (e.g. cDNA) capable of being used to provide such a polypeptide is injected into the animal. If necessary an adjuvant may be administered together with the polypeptide of the present invention. The antibodies can then be purified by virtue of their binding to the polypeptide.

Monoclonal antibodies can be produced from hybridomas. These can be formed by fusing myeloma cells and spleen cells which produce the desired antibody in order to form an immortal cell line. Thus the well-known Kohler & Milstein technique (*Nature* 256 52-55 (1975)) or variations upon this technique can be used.

Techniques for producing monoclonal and polyclonal antibodies which bind to a particular polypeptide are now well developed in the art. They are discussed in standard immunology textbooks, for example in Roitt *et al*, *Immunology* second edition (1989), Churchill Livingstone, London.

In addition to whole antibodies, the present invention includes derivatives thereof which are capable of binding to polypeptides of the present invention. Thus the present invention includes antibody fragments and synthetic constructs. Examples of antibody

fragments and synthetic constructs are given by Dougall *et al* in *Tibtech* 12 372-379 (September 1994).

Antibody fragments include, for example, Fab, F(ab')₂ and Fv fragments (these are discussed in Roitt *et al* [*supra*], for example). Fv fragments can be modified to produce a synthetic construct known as a single chain Fv (scFv) molecule. This includes a peptide linker covalently joining V_H and V_L regions, which contributes to the stability of the molecule. Other synthetic constructs which can be used include CDR peptides. These are synthetic peptides comprising antigen-binding determinants. Peptide mimetics may also be used. These molecules are usually conformationally restricted organic rings which mimic the structure of a CDR loop and which include antigen-interactive side chains.

Synthetic constructs include chimaeric molecules. Thus, for example, humanised (or primatised) antibodies or derivatives thereof are within the scope of the present invention. An example of a humanised antibody is an antibody having human framework regions, but rodent hypervariable regions. Synthetic constructs also include molecules comprising an additional moiety that provides the molecule with some desirable property in addition to antigen binding. For example the moiety may be a label (e.g. a fluorescent or radioactive label). Alternatively, it may be a pharmaceutically active agent.

The antibodies or derivatives thereof of the present invention have a wide variety of uses in addition to their use in purification of polypeptides discussed above.

They can be used in therapy. For example they may be used to block undesirable ligand / receptor or receptor / receptor interactions.

They can be used in diagnosis. For example they may be used in RIAs or ELISAs in order to identify the presence or absence of the type 1 chemokine receptors that are within the scope of the present invention.

5 Nucleic Acid Molecules and Uses Thereof

The present invention also includes nucleic acid molecules within its scope.

Such nucleic acid molecules:

- 10 a) code for a polypeptide according to the present invention; or
 b) are complementary to molecules as defined in a) above; or
 c) hybridise to molecules as defined in a) or b) above.

15 These nucleic acid molecules and their uses will now be discussed in greater detail below:

20 The polypeptides of the present invention can be coded for by a large variety of nucleic acid molecules, taking into account the well-known degeneracy of the genetic code. All of these coding nucleic acid molecules are within the scope of the present invention. They may be administered to an individual and used to express polypeptides of the present invention. Thus they may be used for the same treatments as the polypeptides of the present invention. The nucleic acid molecules may be used directly, e.g. they may be injected into muscle (optionally after being first incorporated into a carrier, e.g. a lipid-based carrier, such as a liposome). Alternatively they may be inserted into vectors.

25 Vectors for use in treatments include replication-deficient adenoviruses, retroviruses or adeno-associated viruses.

Vectors may be used in cloning. They may be introduced into host cells to enable the expression of polypeptides of the present invention using techniques known to the person

skilled in the art. Alternatively, cell free expression systems may be used. By using an appropriate expression system the polypeptides can be produced in a desired form. For example, the polypeptides can be produced by micro-organisms such as bacteria or yeast, by cultured insect cells (which may be baculovirus-infected), by mammalian
5 cells (such as CHO cells) or by transgenic animals that, for instance, secrete the proteins in milk. Where glycosylation is desired, eukaryotic (desirably mammalian) expression systems are preferred.

Polypeptides comprising N-terminal methionine may be produced using certain
10 expression systems, whilst in others the mature polypeptide will lack this residue. Polypeptides may initially be expressed to include signal sequences. Different signal sequences may be provided for different expression systems. Alternatively, signal sequences may be absent.

15 Techniques for cloning, expressing and purifying polypeptides are well known to the skilled person. Various such techniques are disclosed in standard text-books, such as in Sambrook *et al* [*Molecular Cloning* 2nd Edition, Cold Spring Harbor Laboratory Press (1989)]; in Old & Primrose [*Principles of Gene Manipulation* 5th Edition, Blackwell Scientific Publications (1994)]; and in Stryer [*Biochemistry* 4th Edition, W H Freeman
20 and Company (1995)].

In addition to nucleic acid molecules coding for polypeptides of the present invention (referred to herein as "coding" nucleic acid molecules), the present invention also includes nucleic acid molecules complementary thereto. Thus, for example, both strands
25 of a double stranded nucleic acid molecule are included within the scope of the present invention (whether or not they are associated with one another). Also included are mRNA molecules and complementary DNA molecules (e.g. cDNA molecules).

Nucleic acid molecules which can hybridise to one or more of the nucleic acid molecules discussed above are also within the scope of the present invention. Such nucleic acid molecules are referred to herein as "hybridising" nucleic acid molecules.

- 5 A hybridising nucleic acid molecule of the present invention may have a high degree of sequence identity along its length with a nucleic acid molecule within the scope of a) or b) above (e.g. at least 50%, at least 75% or at least 90% sequence identity).

10 As will be appreciated by the skilled person, the greater the degree of sequence identity that a given single stranded nucleic acid molecule has with another nucleic acid molecule, the greater the likelihood that it will hybridise to a nucleic acid molecule which is complementary to that other nucleic acid molecule under appropriate conditions.

15 Desirably hybridising molecules of the present invention are at least 10 nucleotides in length and preferably are at least 25 or at least 50 nucleotides in length.

Preferred hybridising molecules hybridise under stringent hybridisation conditions. One example of stringent hybridisation conditions is where attempted hybridisation is carried out at a temperature of from about 35°C to about 65°C using a salt solution which is about 0.9 molar. However, the skilled person will be able to vary such parameters as appropriate in order to take into account variables such as probe length, base composition, type of ions present, etc.

25 Most preferably, hybridising nucleic acid molecules of the present invention hybridise to a cDNA molecule having the sequence shown in Figure 3 or Figure 5; to an RNA equivalent thereof; or to a complementary sequence to any of the aforesaid molecules.

Hybridising nucleic acid molecules can be useful as probes or primers, for example. Probes can be used to purify and/or to identify nucleic acids. They may be used in

diagnosis. For example probes may be used to determine whether or not an individual has a receptor of the present invention by determining whether or not a complete gene coding for a functional receptor is present. Primers are useful in amplifying nucleic acids or parts thereof, e.g. by PCR techniques.

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In addition to being used as probes or primers, hybridising nucleic acid molecules of the present invention can be used as antisense molecules to alter the expression of polypeptides of the present invention by binding to complementary nucleic acid molecules. (Generally this can be achieved by providing nucleic acid molecules that bind
10 to RNA molecules that would normally be translated, thereby preventing translation due to the formation of duplexes.) This technique can be used in antisense therapy. Antisense molecules may be in the form of DNA or RNA molecules.

15

It is however important to note that nucleic acid molecules for use in the present invention include not only those with classical DNA or RNA structures, but also variants with modified (non-phosphodiester) backbones.. Two successful attempts to replace the entire backbone have been reported - the morpholino derivatives and the peptide nucleic acids (PNAs), which contain an N-(2-aminoethyl)glycine-based pseudopeptide backbone. (See Nielsen, P.E., *Annual Review of Biophysics & Biomolecular Structure*, 24 167-83
20 (1995)). Nucleic acid variants with modified backbones can have increased stability relative to unmodified nucleic acids and are particularly useful where long-term hybridisation is desired (e.g. in antisense therapy).

25

Hybridising molecules may also be provided as ribozymes. Ribozymes can be used to regulate expression by binding to and cleaving RNA molecules that include particular target sequences.

From the foregoing discussion it will be appreciated that a large number of nucleic acids are within the scope of the present invention. Unless the context indicates otherwise,

nucleic acid molecules of the present invention may therefore have one or more of the following characteristics:

- 1) They may be DNA or RNA (including forms with non-naturally occurring bases and/or non-naturally occurring backbones e.g. PNAs).
- 2) They may be single or double stranded.
- 3) They may be provided in recombinant form i.e. covalently linked to a heterologous 5' and/or a 3' flanking sequence to provide a chimaeric molecule (e.g. a vector) which does not occur in nature.
- 4) They may be provided without 5' and/or 3' flanking sequences that normally occur in nature.
- 5) They may be provided in substantially pure form, e.g. by using probes to isolate cloned molecules having a desired target sequence or by using chemical synthesis techniques. (Thus they may be provided in a form which is substantially free from contaminating proteins and/or from other nucleic acids.);
- 6) They may be provided with introns (e.g. as a full-length gene) or without introns (e.g. as cDNA).

The present invention will now be described by way of example only with reference to the accompanying drawings; wherein :

Figure 1 shows an alignment of human and murine receptor amino acid sequences predicted from cDNA sequence information. Identical amino acid residues are boxed in black. The human and murine polypeptides are referred to as hGBRI-ILR and mGBRI-ILR respectively, since they are believed to be interleukin receptors (or at least substantial parts of such receptors).

Figure 2 shows an alignment of the hGBRI-ILR and mGBRI-ILR amino acid sequences shown in Figure 1 with members of the IL-6 type cytokine receptor family within the immunoglobulin domain and haematopoietin receptor module. GCSF-R is granulocyte colony stimulating factor receptor and CNTF-R is ciliary neurotrophic factor.

Figure 3 shows the cDNA sequence obtained for hGBRI-ILR

Figure 4 shows the predicted amino acid sequence obtained from the cDNA sequence provided in Figure 3.

Figure 5 shows the cDNA sequence obtained for mGBRI-ILR

Figure 6 shows the predicted amino acid sequence obtained from the cDNA sequence provided in Figure 5.

Materials and Methods

Cloning of Human and Mouse cDNA for GBRI-ILR

The amino acid sequence N K L C F D D N K L W S D W S E A Q S I G K E Q N from murine the IL-13R was used to search the Genbank database with expressed sequence tags (ESTs) using TBLASTN, in order to identify the relevant EST. BLASTN searches of The Genbank database with this EST sequence allowed the identification of overlapping ESTs. The mouse cDNA clone 479043 was purchased from Research Genetics Inc. (Birmingham, AL). 5'-RACE was used to clone a further 310bp of the murine cDNA upstream of the 5' end of the cDNA clone using the Marathon cDNA amplification kit from Clontech (Palo Alto, CA) on poly A+ RNA extracted from mouse lung following manufacturer's guidelines. The primer used in the PCR amplification (along with the AP-1 primer provided) was

5'-CGTACCACCTCAGCTTGTA CT TG-3'. PCR products were cloned into the vector pCRII (Invitrogen, Leek, The Netherlands) and colonies screened by *colony hybridization* using the oligonucleotide probe 5'-AAGGATCTCACGTGCCGCTGGACACCGGGT-3'.

5 A portion of the human GBRI-ILR cDNA was amplified by PCR using cDNA derived from poly A+ RNA from human lung with the primers 5'-ACCGCCGAGGGCCTCTACTG-3' and 5'-TTGAGGGAGTAGTTGGTGTGGAGG-3'. Amplified products were cloned into the 10 vector pCRII and colonies screened for the relevant insert by *colony hybridization* using the oligonucleotide probe 5'-TGAGCTCTCCCGTGTACTCAACGCCTCCAC-3'. This amplified DNA product was used as a probe to screen a human placental cDNA library in λ gt10. The largest cDNA (1740bp) was recloned into pBluescript II SK-.

15 *DNA and Protein Sequence Analysis*

Sequences were obtained from cDNA clones by both strand automated sequencing, and were analyzed, along with all relevant ESTs, by the sequence analysis software 20 Sequencher (Genecodes Corporation, Ann Arbor, MI). The signalP server (<http://www.cbs.dtu.dk/signalp/cbssignalp.html>) was used to identify the predicted cleavage site of the signal peptide for GBRI-ILR. DNA and amino acid sequence alignments as well as prediction of hydrophobic regions were analyzed with the Wisconsin package version 8.1 (Genetics Computer Group Inc., Madison, WI).

25 *Detection of Human and Mouse mRNA Transcripts for GBRI-ILR*

For detection of human GBRI-ILR mRNA transcripts by Northern blot analysis, the same 310bp cDNA used to screen the human placental library (see above) was used as

a probe. For detection of human gp130 mRNA, a partial cDNA was amplified with the primers 5'-CCGCGCAAGATGTTGACGTT-3' and

5'-CATTCGGACAGCTTGAACAG-3' and used as a probe. For detection of the human IL-6R α , a partial cDNA was amplified with the primers

5'-CTGACCAAGTCTGCCAGGAGACA-3' and

5'-GAGGACCCCACTCACAAACAAC-3' and used as a probe. The Human, Human II, Human Immune System, Human Endocrine and Human Fetal Multiple Tissue Northern Blots (Clontech) were used to detect expression in human tissues. For human cells and cell lines, briefly, poly A+ RNA was isolated using the Oligotex Direct mRNA Mini Kit (Qiagen, Basel, Switzerland) following manufacturers guidelines. 0.5 μ g RNA was resolved on a formaldehyde gel and transferred to a Genescreen membrane (NEN Research Products, Boston, MA). All Northern blots were hybridised with the appropriate probes in ExpressHyb solution (Clontech).

For detection of murine GBRI-ILR transcripts, either cDNA or cRNA probes were used. The cDNA probe was a product of PCR amplification using the primers 5'-CTAGGCTCAGCAAGATCTGATGTCC-3' and

5'-GCTCCAGATTCCCGCCTTTTTCGACC-3'. To generate cRNA probes, the PCR product generated with the primers 5'-CTGGCCCTGGCTAACCTTAATGG-3' and

5'-GCTCCAGATTCCCGCCTTTTTCGACC-3' was cloned into pBluescript II KS- (Stratagene) at the EcoRV restriction site. The plasmid was linearized with the restriction enzyme BlnI and cRNA probes labelled with ³²P by transcription with T3 RNA polymerase from the T3 promoter. The Mouse and Mouse Embryo Multiple Tissue Northern Blots (Clontech), were hybridized with cDNA probe in ExpressHyb solution. For the other Northern blot analysis, mice were immunised with alum precipitated KLH sub-cutaneously and tissues removed at either day 0 or day 14 (with the exception of bone marrow, which was a pool of both). Total RNA was isolated with TRIzol reagent (Life Technologies AG, Basel, Switzerland) and polyA+ RNA was isolated using Oligotex beads (Qiagen). Northern blot analysis was performed

using the cRNA probe on 1.5µg polyA+ RNA as previously described by Gauchat, J-F. *et al*, (European Journal of Immunology, 1989 19:1079). For detection of the murine transcript by RT-PCR, 5µg of total RNA from the appropriate source was reverse transcribed using the first-strand cDNA synthesis kit (Pharmacia LKB Biotechnology, Uppsala, Sweden) following manufacturer's guidelines. PCR was performed using the same primers used to amplify the murine DNA probe (see above). PCR products were transferred to a Genescreen hybridization membrane (NEN research products) and hybridised with 32P labelled oligonucleotide probe 5'-GCGGATCTGGTACTTGGTTTGAAAGAGGAA-3'.

Cloning and Distribution of the Receptor

Cloning of human GBRI-ILR

The Genbank database with expressed sequence tags (ESTs) was searched using TBLASTN with a 20 amino acid sequence surrounding the W-S-x-W-S motif of the mouse IL-13 receptor $\alpha 1$ as query. ESTs showing significant homology were then translated, and the open reading frames used to search the Swissprot database using BLASTP for homologous proteins. The amino acid sequence from the murine EST W66776 showed a high level of homology to members of the IL-6-type cytokine receptor family, as well as to the prolactin receptor. Using the sequence of W66776 to search the Genbank database allowed the identification of overlapping homologous sequences (of both murine and human origin) which in turn were run against the Genbank database to identify more overlapping ESTs (Table I). This allowed the assembly of overlapping nucleic acid sequences encoding the human and mouse putative receptor sequences.

To clone the human cDNA encoding GBRI-ILR, a 310bp PCR product was amplified from human lung cDNA using primers designed from the human ESTs. The PCR product was in turn used as a probe to screen a human placental cDNA library,

allowing the isolation of a full length clone of 1740 bp which included a 3' poly A tail. The human cDNA encoded a precursor protein of 422 amino acids with a putative signal peptide of 37 amino acids. In vitro translation revealed that the AUG codon coding the methionine at the start of the putative signal peptide was indeed used to initiate translation (data not shown).

Cloning of mouse GBRI-ILR

The Genbank database with expressed sequence tags (ESTs) was searched using TBLASTN with a 20 amino acid sequence surrounding the W-S-x-W-S motif of the mouse IL-13 receptor $\alpha 1$ as query. ESTs showing significant homology were then translated, and the open reading frames used to search the Swissprot database using BLASTP for homologous proteins. The amino acid sequence from the murine EST W66776 showed a high level of homology to members of the IL-6-type cytokine receptor family, as well as to the prolactin receptor. Using the sequence of W66776 to search the Genbank database allowed the identification of overlapping homologous sequences (of both murine and human origin) which in turn were run against the Genbank database to identify more overlapping ESTs (Table I). This allowed the assembly of overlapping nucleic acid sequences encoding the human and mouse putative receptor sequences.

The cDNA clone 479043, which gave rise to the mouse EST found furthest 5' in the sequence assembly was obtained from the IMAGE consortium and sequenced and was found to contain an insert of 1 Kb, including a 3' poly A tail. The rapid amplification of 5' cDNA ends (5'-RACE) on murine lung cDNA allowed the cloning of a further 308 bp upstream. The murine cDNA encoded a protein of 383 amino acids. The mouse cDNA sequence was incomplete at the 5' end as the first amino acid of the translated sequence aligned to amino acid 39 of the putative human receptor sequence, and no starting methionine or putative signal peptide could be identified.

Sequence Analysis

Sequence analysis of the human and murine cDNAs showed 85% sequence identity at the nucleic acid level and 96% identity at the amino acid level (Figure1). Amino acid identity between human and mouse gp130 is 77% and between the human and mouse prolactin receptors is 69%. As the level of identity between the human and murine putative receptors is significantly higher, this suggests a functionally important role for the GBRI-ILR. No putative transmembrane domain could be identified in either the human or mouse amino acid sequence, suggesting that the protein encoded by the cloned cDNAs are either secreted or GPI-anchored. As no hydrophobic region at the C terminus of the sequence, characteristic of GPI-anchored proteins such as the CNTF receptor, was identified, it is more likely that the cloned human and mouse cDNAs encode soluble receptors. There are numerous examples of soluble forms of receptors in the type I cytokine receptor family, being the product of either membrane shedding or alternative splicing. These soluble forms can exhibit either antagonistic effects in terms of ligand signalling such as those shown by soluble gp130 and the soluble IL-5 receptor α chain, or agonistic effects, such as those shown by the IL-6 receptor α chain, the CNTF receptor and the IL-11 receptor α chain.

Human and murine GBRI-ILR show close homology to members of the IL-6-type cytokine receptor family (Table II) as well as to the prolactin receptor when used as query to search the SwissProt database. Alignment of the human and mouse amino acid sequences to members of the IL-6-type cytokine receptor family showed regions of conserved homology within the two functionally important cytokine receptor-like domain, most notably at the highly conserved four cysteine residues and the W-S-x-W-S motif (Figure 2). The N-terminal domain of both sequences also appears to represent an Ig-like domain, most closely resembling the C2-set sequence.

One of the human EST's (H14009) showing homology to GBRI-ILR was a sequence derived from a genomic clone (D2-17). This clone was generated by exon

amplification of chromosomal DNA from human chromosome 19p12-13.1, allowing us to localise the human GBRI-ILR gene to this region. The gene for the erythropoietin receptor, which shares significant homology with GBRI-ILR, is the only other member of the receptor family which has been shown to be localized to this arm of chromosome 19.

Distribution of Human and Mouse GBRI-ILR

mRNA expression was studied in human and mouse tissues by Northern blot analysis. The predominantly expressed form of the human mRNA migrated as a 1.7 Kb transcript, a size close to the one predicted from the clone obtained from the library screening. Another transcript of approximately 4.5Kb was seen in several tissues. This form could encode a membrane-anchored form of the receptor, analogous to the two transcripts detected for the IL-5 receptor α chain. Expression of the 1.7Kb transcript could be detected in several tissues, but was less ubiquitous than those of gp130 and IL-6R α . Strongest expression of the human GBRI-ILR mRNA was detected in the spleen, thymus, lymph node, appendix, bone marrow, thyroid, adrenal cortex, stomach, heart, placenta and skeletal muscle. This distribution is compatible with a possible role for human GBRI-ILR in the immune system. Expression was also detected in several cell lines (Table III), such as the fibroblast cell line HEK 293, the monocyte cell line THP-1 (following PMA stimulation), JY lymphoblastoid cells, RPMI 8226 myeloma cells, the mast cell line HMC-1, bronchial epithelial cells HBE-140 and low level expression on HUVEC. In human fetal tissue, a strong expression was seen in the lung, but not in brain, kidney or liver.

In the adult mouse, expression of a 1.7Kb transcript was seen most strongly in the lung, but the transcript could also be detected at lower levels in skeletal muscle as well as heart and brain. Expression of the 1.7Kb transcript was also detected in the lymph node and thymus of immunized and non-immunized mice as well as in mouse bone marrow. In the embryo, the 1.7Kb transcript could first be detected at day 11 post

conception, with expression going through to days 15 and 17 post conception. This pattern of expression would appear to coincide with the emergence of the first detectable progenitors of the immune system, at day 10.5 post conception. Taken together, these data indicate a possible role for GBRI-ILR in the immune system and in embryonic development.

Overcoming cloning / expression difficulties

Several attempts to clone the full length cDNA for both human and mouse GBRI-ILR by PCR failed due to the lack of amplification of a significantly long product when attempting rapid amplification of 5' cDNA ends (5' RACE). This was later found to be due to the presence of a very GC rich region of the DNA at the 5' end of the cDNA, hampering the PCR reaction. This problem was overcome for hGBRI-ILR by screening a placental cDNA library with a cloned cDNA fragment obtained by PCR amplification using primers designed from the human ESTs.

Recombinant protein expression using the baculovirus expression system was also found to be less than efficient when using partial cDNA for hGBRI-ILR which was lacking the immunoglobulin domain. Higher levels of protein production were observed when cDNA encoding the complete N-terminal region of the protein was used.

Table I

Receptor	EST Accession no.	Tissue/Source of Origin
Mouse	AA014965	Placenta
	AA039053	Embryo
	AA049278	Embryo
	AA049280	Embryo
	AA270365	Embryo
	W17583	Embryo
	W66776	Embryo
Human	AA042914	Pregnant uterus
	AA043001	Pregnant uterus
	AA121532	Pregnant uterus
	AA127694	Pregnant uterus
	AA377893	Synovial Sarcoma
	AA406406	Melanocyte/Fetal Heart/uterus
	H14009	Chromosome 19
	N78873	Fetal Lung
	R87407	Brain
	W37175	Fetal Lung
	W46603	Fibroblast
	W46604	Fibroblast

Table II

Cloned Receptor	Alignment With	Identical Residues	Similar Residues
Human	GP130	87/306	120/306
	GCSF	85/306	112/306
	Receptor		
	CNTF	79/306	102/306
	Receptor		
Mouse	IL6 Receptor alpha chain	71/306	104/306
	GP130	89/304	125/304
	GCSF	81/304	115/304

Receptor IL6Receptor alpha chain	71/304	107/304
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Table III

Cell/Cell Line	hGB RI- ILR	Cell/Cell Line	hGB RI- ILR
TT7	-	HL60	-
JURKAT	-	HMC-1	++
JURKAT	-	HBE-140	-/+
(+PMA/Ionomycin)			
JY	+	HBE-140 (IL- 1 β)	+
JY (+IL-4)	++	HBE-140 (TNF α)	+
RPMI 8866	-	HEK 293	++
RPMI 8226	+	HUVEC	-/+
THP-1	-	HUVEC (IL- 1 β /TNF α 2hr)	-/+
THP-1 (+PMA)	+	HUVEC (IL- 1 β /TNF α 8hr)	-/+

5 Example of preparation of recombinant soluble GBRI-ILR

Soluble human GBRI-ILR cDNA, truncated at amino acid 378, and coding for the 6 histidine and 179 recognition tags at the 3' end was cloned into pFASTBAC1. Recombinant virus was produced using the Life Technologies BAC-TO-BAC kit and used to infect SF9 cells expanded in SF900II medium. Protein secreted into the medium was purified using a NI-NTA resin column (which binds the 6 histidines). Purified protein was detected by western blot analysis using a monoclonal antibody recognising the 179 tag (CLEPYTACD).

Example of the generation of hybridomas producing anti-GBRI-ILR monoclonal antibodies

A Balb/c mouse was immunized on day 0, 7 and 28 subcutaneously in the limbs and behind the neck with 100 µg of protein per injection. Three days after the final injection, the draining lymph nodes were obtained and the tissue digested using a collagenase and DNase cocktail according to the procedure reported elsewhere (Kosco-Vilbois M.H., Isolation and Enrichment of Follicular Dendritic Cells from Murine Lymphoid Tissue, *Immunology Methods Manual*, Vol 3, 1997). The resulting cell suspension was resuspended at 10^6 cells per ml and fused with Sp2 myeloma cells using standard "Kohler and Milstein" protocols. The hybridomas were then selected in HAT medium and 7-10 days after fusion, the supernatants harvested for screening.

To screen the hybridoma supernatants, 96 wells plates (Falcon 3912; Becton Dickinson Labware Europe, Meylan, France) were coated overnight at 4°C with 1 µg/ml soluble GBRI-ILR purified from infected Sf9 cell in carbonate buffer pH 9.6. Plates were then washed, blocked with PBS containing 1% BSA and incubated for two hours with 200 µl hybridoma supernatant and washed. GBRI-ILR specific mAbs were revealed using horseradish peroxidase labelled goat anti-mouse IgG (Southern Biotechnology Associates, Inc.) Positive supernatants were retested with plastic immobilized GBRI-ILR. Specificity was checked using an ELISA set up with IL-13Rα1 at 1 µg/ml. The specific positive supernatants were further screened by FACS using HEK-293 cells transfected with a cDNA encoding a fusion protein between GBRI-ILR up to amino acid 354 and the IL-13 Rα1 transmembrane and cytoplasmic regions, IL-13Rα1 cDNA or an empty plasmid (the plasmid used for expression was pEBS). Hybridomas that demonstrated the strongest fluorescence signal on GBRI-ILR transfectants and also did not bind to control proteins or transfectants were retained for further use.

Claims

1. A polypeptide, which:
 - a) has the amino acid sequence of amino acids 38 to 422 shown in Figure 1 for hGBRI-ILR, or which has the amino acid sequence shown in Figure 1 for mGBRI-ILR;
 - b) has one or more amino acid deletions, insertions or substitutions relative to a polypeptide as defined in a) above, but has at least 40% amino acid sequence identity therewith; or
 - c) is a fragment of a polypeptide as defined in a) or b) above, which is at least 10 amino acids long.
2. A polypeptide according to claim 1, which has a haematopoietin receptor module.
3. A polypeptide according to claim 1 or claim 2, which comprises the amino acid sequence of amino acids 38 to 422 shown in Figure 1 for hGBRI-ILR, or the amino acid sequence shown in Figure 1 for mGBRI-ILR.
4. A polypeptide according to any preceding claim, in glycosylated form.
5. A polypeptide according to any preceding claim, in soluble form.
6. A pharmaceutically acceptable composition comprising a polypeptide according to any preceding claim.
7. A polypeptide according to any of claims 1 to 5 or a pharmaceutically acceptable composition according to claim 6, for use in medicine.

8. The use of a polypeptide according to any of claims 1 to 5 in the manufacture of a medicament for treating cancer, an immune disorder, obesity or a developmental disorder.
- 5 9. The use of a polypeptide according to any of claims 1 to 5 in the manufacture of a medicament for treating AIDS, septic shock, embryonic developmental disorders or lung inflammation.
- 10 10. The use of a polypeptide according to any of claims 1 to 5 in screening.
11. The use according to claim 10 in screening for a cytokine that binds to a type 1 cytokine receptor.
- 15 12. The use according to claim 10 in screening for an agonist or antagonist of a cytokine that binds to a type 1 cytokine receptor.
- 20 13. A cytokine that binds to a type 1 cytokine receptor, or an agonist or an antagonist thereof identifiable by or identified by screening as described in any of claims 10 to 12.
- 25 14. A cytokine, an agonist or an antagonist according to claim 13, for use in medicine.
15. The use of a cytokine, an agonist or an antagonist according to claim 12 in the manufacture of a medicament for treating cancer, an immune disorder, obesity or a developmental disorder.
16. The use according to claim 10 in screening for an agonist or antagonist of a cytokine that binds to a type 1 cytokine receptor in the manufacture of a

medicament for treating AIDS, septic shock, embryonic developmental disorders or lung inflammation.

- 5 17. The use of a polypeptide according to any of claims 1 to 5 in raising or selecting antibodies.
18. An antibody or a derivative thereof which binds to a polypeptide according to any of claims 1 to 5.
- 10 19. A pharmaceutically acceptable composition comprising an antibody or a derivative thereof according to claim 18.
20. An antibody or a derivative thereof according to claim 18 or a pharmaceutically acceptable composition according to claim 19, for use in medicine.
- 15 21. The use of an antibody or a derivative thereof according to claim 20 in the preparation of a medicament for treating cancer, an immune disorder, obesity or a developmental disorder.
- 20 22. The use of an antibody or a derivative thereof according to claim 20 in the manufacture of a medicament for treating AIDS, septic shock, embryonic developmental disorders or lung inflammation.
- 25 23. A nucleic acid molecule, which:
a) codes for a polypeptide according to any of claims claim 1 to 5,
b) is complementary to a molecule as defined in a) above, or
c) hybridises to a molecule as defined in a) or b) above.
24. A vector comprising a nucleic acid molecule according to claim 23.

25. A host comprising a nucleic acid molecule according to claim 23 or a vector according to claim 24.
- 5 26. A method for obtaining a polypeptide according to any of claims 1 to 5, comprising incubating a host according to claim 25 under conditions causing expression of said polypeptide and then purifying said polypeptide.
- 10 27. A nucleic acid molecule, vector or host according to any of claims 23, 24 or 25 respectively, for use in medicine.
28. The use of a nucleic acid molecule, vector or host according to any of 23, 24 or 25 respectively in the preparation of a medicament for antisense therapy.
- 15 29. The use of nucleic acid molecule according to claim 23 as a probe or as a primer.
30. The invention as substantially hereinbefore described.

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mGBRI-ILR :
hGBRI-ILR : MPAGRRGPAAQSAARRPPPLPLLLLLCVLGAPRAGSGAH-----TAVISPODPTLLIGSSLLQATCSHFGDTPCATAEGLYVTTPNGRRLPSELRRLENSTLALALANL : 64
 : 103

mGBRI-ILR :
hGBRI-ILR : NGSROOSGDNLVCHARDGSSILAGPCLYLDLPPPERHFNISCSWRRNRKDDTCRTTPGAHGETFLHTNYSIKYKURWYGQDNTCEBYHTVGPHSCHIPKDLALFTP : 167
 : 206

mGBRI-ILR :
hGBRI-ILR : NGSRCRSGDNLVCHARDGSSILAGSCLYVCLPPPERHFNISCSWRRNRKDDTCRTTPGAHGETFLHTNYSIKYKURWYGQDNTCEBYHTVGPHSCHIPKDLALFTP : 167
 : 206

mGBRI-ILR :
hGBRI-ILR : YEIVVEATNRLGSAKRSVDLTLEVLDDVVTTLUPPPDDVHVSRVGGLEDOLSVRWVSPPALKDFLFOAKYQIRYRVEDSVDDWKVDDVSNOTSCRLAGLRKPTVYEV : 270
 : 309

mGBRI-ILR :
hGBRI-ILR : QVRCNPFGIYGSRRKAGINSEMSHPTAASTPRSERPGPGGCVCEPRGGEPSSGPPVRRRELKQFLGWLKRRHAYCSNLSFRLYDQWRAMWOKSHKTRNODGILPSC : 373
 : 412

mGBRI-ILR :
hGBRI-ILR : RRGARGPAG : 383
 : 422

FIG. 1

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FIG. 3

5
1 CGCCCAGCGA CGTGCGGGCG GCCTGGCCCG CGCCCTCCCG CGCCCGGCCT
51 GCGTCCCGCG CCCTGCGCCA CCGCCGCCGA GCCGCAGCCC GCCGCGCGCC
101 CCCGGCAGCG CCGGCCCCAT GCCCGCCGCG CGCCGGGGCC CCGCCGCCCA
10 151 ATCCGCGCGG CGGCCGCCG CGTTGCTGCC CCTGCTGCTG CTGCTCTGCG
201 TCCTCGGGGC GCCGCGAGCC GGATCAGGAG CCCACACAGC TGTGATCAGT
251 CCCCAGGATC CCACGCTTCT CATCGGCTCC TCCCTGCTGG CCACCTGCTC
15 301 AGTGCACGGA GACCCACCAG GAGCCACCGC CGAGGGCCTC TACTGGACCC
351 TCAACGGGCG CCGCCTGCCC CCTGAGCTCT CCCGTGTACT CAACGCCTCC
20 401 ACCTTGGCTC TGGCCCTGGC CAACCTCAAT GGGTCCAGGC AGCGGTGCGG
451 GGACAACCTC GTGTGCCACG CCCGTGACGG CAGCATCCTG GCTGGCTCCT
501 GCCTCTATGT TGGCCTGCCC CCAGAGAAAC CCGTCAACAT CAGCTGCTGG
25 551 TCCAAGAACA TGAAGGACTT GACCTGCCGC TGGACGCCAG GGGCCCACGG
601 GGAGACCTTC CTCCACACCA ACTACTCCCT CAAGTACAAG CTTAGGTGGT
30 651 ATGGCCAGGA CAACACATGT GAGGAGTACC ACACAGTGGG GCCCCACTCC
701 TGCCACATCC CCAAGGACCT GGCTCTCTTT ACGCCCTATG AGATCTGGGT
751 GGAGGCCACC AACCGCCTGG GCTCTGCCCC CTCCGATGTA CTCACGCTGG
35 801 ATATCCTGGA TGTGGTGACC ACGGACCCCC CGCCCGACGT GCACGTGAGC
851 CGCGTCGGGG GCCTGGAGGA CCAGCTGAGC GTGCGCTGGG TGTCGCCACC
40 901 CGCCCTCAAG GATTTCTCTT TTCAAGCCAA ATACCAGATC CGCTACCGAG
951 TGGAGGACAG TGTGGACTGG AAGGTGGTGG ACGATGTGAG CAACCAGACC
1001 TCCTGCCGCC TGGCCGGCCT GAAACCCGGC ACCGTGTACT TCGTGCAAGT
45 1051 GCGCTGCAAC CCCTTTGGCA TCTATGGCTC CAAGAAAGCC GGGATCTGGA
1101 GTGAGTGGAG CCACCCACCA GCCGCCTCCA CTCCCCGAG TGAGCGCCCC
50 1151 GGCCCGGGCG GCGGGGCGTG CGAACCGCGG GCGGAGAGC CGAGCTCGGG

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1201 GCCGGTGGCG CGCGAGCTCA AGCAGTTCCT GGGCTGGCTC AAGAAGCACG
1251 CGTACTGCTC CAACCTCAGC TTCCGCCTCT ACGACCACTG GCGAGCCTGG
5 1301 ATGCAGAAGT CGCACAAAGAC CCGCAACCAG GACGAGGGGA TCCTGCCCTC
1351 GGGCAGACGG GGCACGGCGA GAGGTCCTGC CAGATAAGCT GTAGGGGCTC
1401 AGGCCACCCT CCCTGCCACG TGGAGACGCA GAGGCCGAAC CCAAACCTGGG
10 1451 GCCACCTCTG TACCCTCACT TCAGGGCACC TGAGCCACCC TCAGCAGGAG
1501 CTGGGGTGGC CCCTGAGCTC CAACGGCCAT AACAGCTCTG ACTCCACAGT
15 1551 GAGGCCACCT TTGGGTGCAC CCCAGTGGGT GTGTGTGTGT GTGTGAGGGT
1601 TGGTTGAGTT GCCTAGAACC CCTGCCAGGG CTGGGGGTGA GAAGGGGAGT
1651 CATTACTCCC CATTACCTAG GGCCCTCCA AAAGAGTCCT TTAAATAAAA
20 1701 TGAGCTATTT AGGTGC

FIG. 3 CONT'D

1 MPAGRRGPAA QSARRPPPLL PLLLLLCVLG APRAGSGAHT AVISPQDPTL
5 51 LIGSSLLATC SVHGDPPGAT AEGLYWTLNG RRLPPELSRV LNA STLALAL
101 ANLNGSRQRS GDNLVCHARD GSILAGSCLY VGLPPEKPVN ISCWSKNMKD
10 151 LTCRWTPGAH GETFLHTNYS LKYKLRWYGQ DNTCEEYHTV GPHSCHIPKD
201 LALFTPYEIW VEATNRLGSA RSDVLTLDIL DVVTDPFPD VHVS RVGGLE
251 DQLSVRWVSP PALKDFLFQA KYQIRYRVED SVDWKVVDDV SNQTSCLAG
15 301 LKPGTVYFVQ VRCNPFGIYG SKKAGIWSEW SHPTAASTPR SERPGPGGGA
351 CEPRGGEPS GPVRRELKQF LGWLKKHAYC SNLSFRLYDQ WRAWMQKSHK
20 401 TRNQDEGILP SGRRGTARGP AR

FIG. 4

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1301 GTCACACTTG GATATACCCC AGTGTGGGTA GGGTTGGGGT ATTGCAGGGC
1351 CTCCCAAGAG TCTCTTTAAA TAAATAAAGG AGTTGTTTCTAG TCCCGAGAAA
1401 AAAAAAAAAA AAAAAAATTT CCGCGGCCGC

FIG. 5CONT'D

5 1 TAVISPDPT LIGSSSLQAT CSINGDTPGA TAEGLYWTLN GRRLPSELSR
51 LLNTSTLALA LANLNGSRQQ SGDNLVCHAR DGSILAGSCL YVGLPPEKPF
10 101 NISCWSRNMK DLTCRWTPGA HGETFLHTNY SLKYKLRWYG QDNTCEEYHT
151 VGPHSCHIPK DLALFTPYEI WVEATNRLGS ARSDVLTLDV LDVVTDDPPP
201 DVHVS RVGGL EDQLSVRWVS PPALKDFLFQ AKYQIRYRVE DSVDWKVVDD
15 251 VSNQTSCRLA GLKPGTVYFV QVRCNPFGIY GSKKAGIWSE WSHPTAASTP
301 RSERPGPGGG VCEPRGGEPS SGPVRRELKQ FLGWLKKHAY CSNLSFRLYD
20 351 QWRAWMQKSH KTRNQDEGIL PSGRGAARG PAG*TLRIGH PPAGSDLEAH
401 LNWSPSVPSG QQRNLPEAGA Q

FIG. 6